Supplementary Data Files

Supplementary Materials and Methods

EmrE variant protein purification from E. coli membranes. To verify that each plasmid expressed and accumulated EmrE variant protein in *E. coli* JW0451 cell membranes, an organic extraction and reverse phase gel filtration purification procedure was used as described in Winstone *et al*. 2002 and Bay et al. 2012 [1,2]. An organic extraction technique best method to purify un-tagged EmrE proteins based on its small size (12 kDa) and intrinsically high hydrophobicity [3]. Attempts to accurately quantify EmrE protein variants from isolated *E. coli* whole membrane preparations using SDS-Tricine polyacrylamide gel electrophoresis (PAGE) alone was fraught with reproducible difficulties due to the small size of this protein (12 kDa) overlapping with other bands at 13-15 kDa and the technical challenges estimating the time necessary to completely 'run-off' the loading dye front to resolve ≤14 kDa band regions at the bottom of the gel (Figure S5E). 'Leaky' expression from the pMS119EH P*tac* promoter was sufficient to determine a drug resistance phenotype and MIC values in this study but was insufficient to detect protein accumulation in the membranes (Figure S5E), therefore, isopropyl β-D-1-thiogalactopyranoside (IPTG) induction was required to detect the presence of each EmrE variant.

EmrE variant accumulation within *E.coli* membranes was confirmed using a chloroform: methanol (3:1) organic extraction and reverse phase chloroform: methanol (1:1) gel filtration purification method as described by [2]. The only exception to this procedure was the volumes of starting cultures used to extract and purify EmrE protein variants: 10 mL of 37ºC shaken overnight cultures of each *E. coli* K12 JW0451 plasmid transformant were separately diluted 10^{-3} into fresh 500 mL flasks of LB containing 100 µg/mL ampicillin (final concentration) and shaken at 37ºC until an $OD_{600\ nm}$ = 0.5 units was attained. Cultures were induced to over-express their plasmid encoded *emrE* variants by the addition of 0.1 mM IPTG (final concentration) and incubated for another 3 hrs at 37ºC with shaking. JW0451 strains with parental vector (pMS119EH) were included as background EmrE accumulation controls. Cells were harvested by centrifugation, washed then frozen at -80ºC, membrane isolation and organic extraction followed by reverse phase gel filtration were performed as described by [2]. Reverse phase gel filtration (Sephadex LH-20) was used to separate EmrE variant protein fractions from the second eluted peak (1 mL fractions 16-20) as described in [2]. All fractions were dried under nitrogen gas in a single vial and resuspended in 1% SDS buffered in 50 mM phosphate buffer pH 7.0. The concentration of EmrE was determined by UV absorbance spectrophotometry at 280 nm and each EmrE protein variant was separated by 12% (T) SDS-Tricine PAGE as described in [2]. 10 µL of 5 µM EmrE variant protein samples were added to 5 µL SDS loading dye (50mM Tris, 3% w/v SDS, 200 mM DTT, 20% w/v glycerol and 0.2% w/v bromophenol blue, pH 7.0). Protein bands were visualized by adding trichloroethanol into the gels as described in [2]. Gel images of each extracted EmrE variant protein are shown in Figure S5A-D.

Supplementary Figures

Figure S1. Chemical structures of all 18 QCCs examined in this study.

Figure S2. A hierarchical agglomerative cluster heatmap of EmrE variant MIC values determined for 17 different QCCs. The fold change in MIC for values obtained for EmrE variants (y-axis)

exposed to each QCC tested (x-axis) is shown on the heatmap (refer to panel legend). The black dividing line on the heatmap indicates the division between EmrE variant clusters V1 and V2 and lettering indicates nodes discussed in text. Each EmrE variant is listed on the right-hand y-axis and colored according to its TM position. All 17 QCCs are listed on the bottom x-axis along with corresponding heatmap (shades of purple) that indicates specific chemical structural features for each drug. Dendrograms on the left y-axis and top x-axis show cluster associations between EmrE variant MICs and QCCs and clusters discussed in text are labelled according to cluster: EmrE variant clusters (V1-V2) and QCC clusters (Q1-Q3).

Figure S3. The location of conserved EmrE variants specific for each of the 17 QCCs tested and mapped onto helical wheel projections of the highest resolved structure of dimeric EmrE. 17 different top-down views of helical wheel projections of EmrE dimers are shown from Figure 2. Each dimer highlights the residues in each monomer (A and B) that are colored according to the fold change MIC values from the WT (refer to fold change legend in bottom right Figure panel) for a particular QCC listed in the grey circle. Each grey circle indicates the QCC tested and binding pocket of the dimer based on the electron density of bound TPP from the original EmrE structure.

Figure S4. An excel file of replicate raw data collected for *E. coli* JW0451 strains transformed with plasmids encoding various EmrE variants tested in this study.

Figure S5. SDS-Tricine PAGE analysis of EmrE protein variants tested in this study to confirm variant protein accumulation/ expression. Panels **A-D** show 12% (T) SDS-Tricine PAGE separations of each EmrE protein variant in this study in 1% SDS 50 mM phosphate buffer solution where 5 µM protein (10 µL sample volume) was loaded in each well. Each EmrE variant protein is labelled according to its single letter amino acid replacement as listed on Table 2. Wells labelled 'vector' indicate organic extracted reverse phase gel filtration purified membranes from strains expressing only the parental pMS119EH vector (from pooled fractions 16-20; no EmrE protein was detectable by Abs 280 nm or visible by PAGE). **Panel E** shows the 16% (T) SDS-Tricine PAGE separation of 1 mg/ml protein crude whole membrane isolations of *E. coli* JW0451 transformants over-expressing the parental vector (vector), wildtype *emrE* (WT), and *emrE* E14A (E14A) in the presence and absence of 0.1 mM IPTG (+/- IPTG). Note the absence of any 12 kDa band in membrane isolations over-expressing the parental vector (vector+IPTG) or the uninduced WT (WT-IPTG) as compared to cells over-expressing WT *emrE* (WT+IPTG). In panels **A-B** 5 µl of Bio-Rad low molecular weight protein standard (Ladder 1) and in panels C-E, 5 µL of Bio-Rad low molecular weight protein and polypeptide standards (Ladder 2) were used as protein size standards. All bands were visualized by 0.5% w/v trichloroethanol addition to the cast gels after 3 min of ultraviolet illumination.

Supplementary References

[1] T.L. Winstone, K.A. Duncalf, R.J. Turner, Optimization of expression and the purification by organic extraction of the integral membrane protein EmrE, Protein Expr Purif. 26 (2002) 111–121.

[2] D.C. Bay, R.J.J. Turner, Spectroscopic analysis of small multidrug resistance protein EmrE in the presence of various quaternary cation compounds, BBA - Biomembr. 1818 (2012) 1318–1331.

[3] H. Yerushalmi, M. Lebendiker, S. Schuldiner, EmrE, an *Escherichia coli* 12-kDa multidrug transporter, exchanges toxic cations and H+ and is soluble in organic solvents, J Biol Chem. 270 (1995) 6856–6863.

[4] H.A. de Boer, L.J. Comstock, M. Vasser, The tac promoter: a functional hybrid derived from the trp and lac promoters, Proc Natl Acad Sci U S A. 80 (1983) 21–25.

Table S1. Primer sequence pairs used to generate *emrE* variants using PCR based site-directed mutagenesis.

***emrE* codon replacement**	**Forward (F) and reverse (R) primer sequences**
L7A	F: 5'GAACCCTTATATTTAT**GCA**GGTGGTGCAATACTTGC 3' R: 5' GCAAGTATTGCACCACC**TGC**ATAAATATAAGGGTTC 3'
A10C	F: 5'CCTTATATTTATCTTGGTGGT**TGT**ATACTTGCAGAGGTC 3' R: 5'GACCTCTGCAAGTAT**ACA**ACCACCAAGATAAATATAAGG 3'
I11C	F: 5'CTTGGTGGTGCA**TGT**CTTGCAGAGGTCATTGGTACAACC 3' R: 5'GGTTGTACCAATGACCTCTGCAAG**ACA**TGCACCACCAAG 3
E14D	F: 5'GTGGTGCAATACTTGCA**GAC**GTCATTGGTACAACC3' R: 5'GGTTGTACCAATGAC**GTC**TGCAAGTATTGCACCAC3'
E14A	F: 5'GTGGTGCAATACTTGCA**GCG**GTCATTGGTACAACC3' R: 5'GGTTGTACCAATGAC**CGC**TGCAAGTATTGCACCAC3'
V15C	F: 5'GTGGTGCAATACTTGCAGAG**TGT**ATTGGTACAACCTTAATG3' R: 5'CATTAAGGTTGTACCAAT**ACA**CTCTGCAAGTATTGCACCAC3'
G17A	F: 5'CTTGCAGAGGTCATT**GCG**ACAACCTTAATGAAGTTTC3' R: 5'GAAACTTCATTAAGGTTG**TCG**CAATGACCTCTGCAAG3'
T18C	F: 5'CTTGCAGAGGTCATTGGT**TGT**ACCTTAATGAAGTTTTCAG 3' R: 5' CTGAAAACTTCATTAAGGT**ACA**ACCAATGACCTCTGCAAG 3'
M21C	F:5'CATTGGTACAACCTTA**TGT**AAGTTTTCAGAAGGTTTTACACGG 3' R:5'CCGTGTAAAACCTTCTGAAAACTT**ACA**TAAGGTTGTACCAATG 3'
G26C	F: 5'CCTTAATGAAGTTTTCAGAA**TGT**TTTACACGGTTATGGCC 3' R: 5' GGCCATAACCGTGTAAA**ACA**TTCTGAAAACTTCATTAAGG 3'
F27C	F: 5'GAAGTTTTCAGAAGGT**TGT**ACACGGTTATGGCCATCTG 3' R: 5'CAGATGGCCATAACCGTGT**ACA**ACCTTCTGAAAACTTC 3'
W31A	F: 5'GAAGGTTTTACACGGTTA**GCA**CCATCTGTTGGTAC 3' R: 5'GTACCAACAGATGG**TGC**TAACCGTGTAAAACCTTC 3'
P32C	F: 5' GGTTTTACACGGTTATGG**TGT**TCTGTTGGTACAATTATTTG 3' R: 5'CAAATAATTGTACCAACAGA**ACA**CCATAACCGTGTAAAACC 3'
Y40A	F: 5'CTGTTGGTACAATTATTTGTGCATGT**GCA**TCATTCTGG 3' R: 5'CCAGAATGATGCACA**TGC**ACAAATAATTGTACCAACAG 3'
F44C	F: 5' GTTATTGTGCATCA**TGT**TGGTTATTAGCTCAGACGCTGG 3' R: 5'CCAGCGTCTGAGCTAATAACCA**ACA**TGATGCACAATAAC 3'
W45A	F: 5' GTTATTGTGCATCATTC**GCA**TTATTAGCTCAGACGCTG 3' R: 5'CAGCGTCTGAGCTAATAA**TGC**GAATGATGCACAATAAC 3'
L47C	F: 5'GCATCATTCTGGTTA**TGT**GCTCAGACGCTGGCTTATATTCC 3' R: 5'GGAATATAAGCCAGCGTCTGAGC**ACA**TAACCAGAATGATGC 3'
P55C	F: 5'GCTCAGACGCTGGCTTATATT**TGT**ACAGGGATTGCTTATGC 3' R: 5'GCATAAGCAATCCCTGT**ACA**AATATAAGCCAGCGTCTGAGC 3'
G57C	F: 5'CTGGCTTATATTCCTACA**TGT**ATTGCTTATGCTATCTGG 3' R: 5'CCAGATAGCATAAGCAAT**ACA**TGTAGGAATATAAGCCAG 3'
A59C	F: 5'CTTATATTCCTACAGGGATT**TGT**TATGCTATCTGGTC 3' R: 5'GACCAGATAGCATA**ACA**AATCCCTGTAGGAATATAAG 3'
Y60A	F: 5'CCTACAGGGATTGCT**GCA**GCTATCTGGTCAGGAGTC 3' R: 5'GACTCCTGACCAGATAGC**TGC**AGCAATCCCTGTAGG 3'
I62C	F: 5'CAGGGATTGCTTATGCT**TGT**TGGTCAGGAGTCGG 3' R: 5'CCGACTCCTGACCA**ACA**AGCATAAGCAATCCCTG 3'
Table S1 continued	

***emrE* codon replacement**	**Forward (F) and reverse (R) primer sequences**
W63A	F: 5'GGATTGCTTATGCTATC**GCA**TCAGGAGTCGGTATTG 3' R: 5'CAATACCGACTCCTGA**TGC**GATAGCATAAGCAATCC 3'
S64C	F: 5'GCTTATGCTATCTGG**TGT**GGAGTCGGTATTGTCCTG 3' R: 5'CAGGACAATACCGACTCC**ACA**CCAGATAGCATAAGC 3'
V66C	F: 5'GCTTATGCTATCTGGTCAGGA**TGT**GGTATTGTCCTGATTAG 3' R: 5'CTAATCAGGACAATACC**ACA**TCCTGACCAGATAGCATAAGC 3'
V69C	F: 5'GGTCAGGAGTCGGTATT**TGT**CTGATTAGCTTACTG 3' R: 5'CAGTAAGCTAATCAG**ACA**AATACCGACTCCTGACC 3'
S72C	F:5'GGTCAGGAGTCGGTATTGTCCTGATT**TGT**TTACTGTCATGGG 3' R:5'CCCATGACAGTAA**ACA**AATCAGGACAATACCGACTCCTGACC 3'
W76A	F: 5'CTGATTAGCTTACTGTCA**GCA**GGATTTTTCGGCCAAC 3' R: 5'GTTGGCCGAAAAATCC**TGC**TGACAGTAAGCTAATCAG 3'
G90C	F: 5'CTGGACCTGCCAGCCATTATA**TGT**ATGATGTTGATTTGTG 3' R: 5' CACAAATCAACATCAT**ACA**TATAATGGCTGGCAGGTCCAG 3'
L93C	F: 5'CATTATAGGCATGATG**TGT**ATTTGTGCCGGTGTGTTG 3' R: 5'CAACACACCGGAACAAAT**ACA**CATCATGCCTATAATG 3'
I94C	F: 5'GCCATTATAGGCATGATGTTGTGT**TGT**GCCGGTGTGTTG 3' R: 5'CAACACACCGGCACA**ACA**CAACATCATGCCTATAATGGC 3'
G97C	F: 5'GATGTTGATTTGTGCC**TGT**GTGTTGATTATTAATTTATTG 3' R: 5'CAATAAATTAATAATCAACAC**ACA**GGCACAAATCAACATC 3'
L103C	F: 5'GTGTTGATTATTAAT**TGT**TTGTCACGAAGCACACC 3' R: 5'GGTGTGCTTCGTGACAA**ACA**ATTAATAATCAACAC 3'
S105C	F: 5'GTTGATTATTAATTTATTG**TGT**CGAAGCACACCAC 3' R: 5'GTGGTGTGCTTCG**ACA**CAATAAATTAATAATCAAC 3'
P*tac* promoter**	F: 5'CTGTTGACAATTAATCATCGGCTCGTATAATG 3'

*Bold underlined nucleotides indicate the site of Cysteine (TGT) or Alanine (GCA) codon replacement.
** Sequencing primer for the *PtacI* promoter region in pMS119EH used to sequence *emrE* codon replacement variants generated in this study [4].

Table S2. A summary of mean MIC values for 18 QCC determined in *E.coli* JW0451 strain at 10^{-2} dilution expressing each of the 33 plasmid encoded EmrE variants used in this study.

	MIC (in μg/ml) of QCC																	
EmrE variant	**ACR**	**PRO**	**CV**	**R6G**	**PY**	**ET**	**TPA**	**MTP**	**TPP**	**DQ**	**MV**	**BZ**	**MAC**	**STAC**	**CPC**	**CPB**	**CAC**	**CET**
***negative**	16	16	8	4	2	16	8	60	16	16	250	8	8	500	4	4	64	4
WT	64	128	32	32	8	32	32	120	32	500	500	16	16	500	8	8	128	16
L7A	32	128	64	32	4	32	32	120	16	16	250	8	16	500	8	8	64	16
A10C	16	64	32	32	2	32	32	60	32	8	64	16	16	250	8	8	32	4
I11C	128	128	32	32	32	32	32	120	32	500	500	16	16	500	8	8	128	8
E14D	8	32	16	32	4	16	16	60	16	8	64	8	16	250	4	4	64	4
E14A	---	---	---	4	---	8	---	---	---	4	32	---	4	---	---	---	---	---
V15C	128	256	32	8	32	32	32	120	32	500	500	16	16	500	8	8	128	16
G17A	128	64	64	32	16	32	32	120	16	8	250	16	16	250	16	8	128	16
T18C	64	64	4	64	4	16	32	120	32	500	125	16	16	250	16	8	64	16
M21C	64	128	4	8	4	16	32	120	32	32	500	16	16	500	8	8	128	8
G26C	64	128	8	8	4	16	32	120	32	16	500	16	16	500	8	4	128	8
F27C	64	128	4	8	4	32	16	120	32	64	500	16	16	500	8	4	128	8
W31A	64	64	64	32	16	64	16	120	16	16	500	16	16	250	8	8	64	8
P32C	128	256	32	32	32	32	32	120	32	500	500	16	16	500	8	8	128	16
Y40A	128	64	16	32	8	64	32	120	8	8	500	16	32	250	8	8	128	16
F44C	64	128	4	8	2	32	32	60	32	16	250	8	16	500	8	4	128	4
W45A	64	128	32	32	16	64	32	120	16	8	500	8	16	250	8	8	64	8
L47C	16	64	8	16	1	16	32	120	32	16	32	8	16	500	8	4	128	4
P55C	64	128	8	4	2	32	16	60	16	500	500	16	16	500	4	4	128	4
G57C	64	128	4	16	2	32	32	120	32	500	125	16	16	500	8	8	64	8
A59C	32	128	16	32	4	64	32	60	16	16	500	8	16	500	8	8	64	8
Y60A	32	64	32	32	8	32	32	120	16	16	250	8	16	250	16	8	64	8
I62C	64	128	64	32	4	128	32	120	8	16	250	16	16	500	8	8	64	8
W63A	16	32	32	16	2	16	16	240	8	500	64	16	16	500	16	16	128	16
S64C	64	128	32	32	8	64	16	60	16	16	128	8	16	500	8	8	64	8
V66C	128	128	32	32	16	64	32	120	16	16	250	16	16	250	8	8	128	8
V69C	128	128	32	32	16	64	16	60	32	8	500	8	16	500	8	8	128	8
S72C	64	128	32	32	16	64	32	120	32	16	500	8	16	250	8	8	64	16
W76A	64	64	16	16	8	32	16	60	8	16	500	4	16	250	8	8	128	16
G90C	64	64	4	16	2	32	32	120	32	64	250	8	16	500	8	8	128	8
L93C	256	128	32	32	16	64	32	120	16	16	500	8	16	250	8	8	128	8
I94C	32	64	32	16	2	16	32	240	8	500	250	8	16	500	8	8	64	16
G97C	16	64	32	32	2	32	32	60	32	8	250	8	8	500	8	8	64	4
L103C	64	128	32	32	8	64	32	120	16	16	500	8	32	500	8	8	64	8
S105C	64	128	32	32	4	128	32	60	16	16	500	8	16	500	8	8	64	4

Mean values reported in this table were derived from MIC data provided in Figure S4.

*****Indicates MIC values for JW0451 transformed with the negative control pMS119EH plasmid only.

--- Indicates untested compounds.

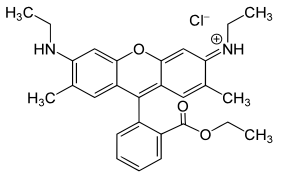
Abbreviations: acriflavin (ACR), proflavin (PRO), crystal violet (CV), rhodamine 6G (R6G), pyronin Y (PY), ethidium bromide (ET), tetraphenylarsonium chloride (TPA), methyltriphenyl phosphonium bromide (MTP), tetraphenylphosphonium chloride (TPP), dequalinium dichloride (DQ), methyl viologen dichloride (MV), benzalkonium chloride (BZ), myristalkonium chloride (MAC), cetylpyridinium chloride (CPC), cetylpyridinium bromide (CPB), cetalkonium chloride (CAC), stearyltrimethylammonium chloride (STAC) and cetrimide (CET).

Table S3. A summary of calculated mean fold change MIC values for each EmrE variant relative to the WT for 17 QCCs*.

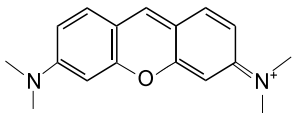
	MIC fold change relative to the WT																
EmrE var-iant	**ACR**	**PRO**	**CV**	**R6G**	**PY**	**ET**	**TPA**	**MTP**	**TPP**	**DQ**	**MV**	**BZ**	**MAC**	**CAC**	**CPB**	**CPC**	**CET**
L7A	0.50	1.00	2.00	1.00	0.50	1.00	1.00	1.00	0.50	0.03	0.50	0.50	1.00	1.00	1.00	0.50	1.00
A10C	0.25	0.50	1.00	1.00	0.25	1.00	1.00	0.50	1.00	0.02	0.13	1.00	1.00	1.00	1.00	0.25	0.25
I11C	2.00	1.00	1.00	1.00	4.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50
E14D	0.13	0.25	0.50	1.00	0.50	0.50	0.50	0.50	0.50	0.02	0.13	0.50	1.00	0.50	0.50	0.50	0.25
V15C	2.00	2.00	1.00	0.25	4.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G17A	2.00	0.50	2.00	1.00	2.00	1.00	1.00	1.00	0.50	0.02	0.50	1.00	1.00	2.00	1.00	1.00	1.00
T18C	1.00	0.50	0.13	2.00	0.50	0.50	1.00	1.00	1.00	1.00	0.25	1.00	1.00	2.00	1.00	0.50	1.00
M21C	1.00	1.00	0.13	0.25	0.50	0.50	1.00	1.00	1.00	0.06	1.00	1.00	1.00	1.00	1.00	1.00	0.50
G26C	1.00	1.00	0.25	0.25	0.50	0.50	1.00	1.00	1.00	0.03	1.00	1.00	1.00	1.00	0.50	1.00	0.50
F27C	1.00	1.00	0.13	0.25	0.50	1.00	0.50	1.00	1.00	0.13	1.00	1.00	1.00	1.00	0.50	1.00	0.50
W31A	1.00	0.50	2.00	1.00	2.00	2.00	0.50	1.00	0.50	0.03	1.00	1.00	1.00	1.00	1.00	0.50	0.50
P32C	2.00	2.00	1.00	1.00	4.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Y40A	2.00	0.50	0.50	1.00	1.00	2.00	1.00	1.00	0.25	0.02	1.00	1.00	2.00	1.00	1.00	1.00	1.00
F44C	1.00	1.00	0.13	0.25	0.25	1.00	1.00	0.50	1.00	0.03	0.50	0.50	1.00	1.00	0.50	1.00	0.25
W45A	1.00	1.00	1.00	1.00	2.00	2.00	1.00	1.00	0.50	0.02	1.00	0.50	1.00	1.00	1.00	0.50	0.50
L47C	0.25	0.50	0.25	0.50	0.13	0.50	1.00	1.00	1.00	0.03	0.06	0.50	1.00	1.00	0.50	1.00	0.25
P55C	1.00	1.00	0.25	0.13	0.25	1.00	0.50	0.50	0.50	1.00	1.00	1.00	1.00	0.50	0.50	1.00	0.25
G57C	1.00	1.00	0.13	0.50	0.25	1.00	1.00	1.00	1.00	1.00	0.25	1.00	1.00	1.00	1.00	0.50	0.50
A59C	0.50	1.00	0.50	1.00	0.50	2.00	1.00	0.50	0.50	0.03	1.00	0.50	1.00	1.00	1.00	0.50	0.50
Y60A	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.03	0.50	0.50	1.00	2.00	1.00	0.50	0.50
I62C	1.00	1.00	2.00	1.00	0.50	4.00	1.00	1.00	0.25	0.03	0.50	1.00	1.00	1.00	1.00	0.50	0.50
W63A	0.25	0.25	1.00	0.50	0.25	0.50	0.50	2.00	0.25	1.00	0.13	1.00	1.00	2.00	2.00	1.00	1.00
S64C	1.00	1.00	1.00	1.00	1.00	2.00	0.50	0.50	0.50	0.03	0.26	0.50	1.00	1.00	1.00	0.50	0.50
V66C	2.00	1.00	1.00	1.00	2.00	2.00	1.00	1.00	0.50	0.03	0.50	1.00	1.00	1.00	1.00	1.00	0.50
V69C	2.00	1.00	1.00	1.00	2.00	2.00	0.50	0.50	1.00	0.02	1.00	0.50	1.00	1.00	1.00	1.00	0.50
S72C	1.00	1.00	1.00	1.00	2.00	2.00	1.00	1.00	1.00	0.03	1.00	0.50	1.00	1.00	1.00	0.50	1.00
W76A	1.00	0.50	0.50	0.50	1.00	1.00	0.50	0.50	0.25	0.03	1.00	0.25	1.00	1.00	1.00	1.00	1.00
G90C	1.00	0.50	0.13	0.50	0.25	1.00	1.00	1.00	1.00	0.13	0.50	0.50	1.00	1.00	1.00	1.00	0.50
L93C	4.00	1.00	1.00	1.00	2.00	2.00	1.00	1.00	0.50	0.03	1.00	0.50	1.00	1.00	1.00	1.00	0.50
I94C	0.50	0.50	1.00	0.50	0.25	0.50	1.00	2.00	0.25	1.00	0.50	0.50	1.00	1.00	1.00	0.50	1.00
G97C	0.25	0.50	1.00	1.00	0.25	1.00	1.00	0.50	1.00	0.02	0.50	0.50	0.50	1.00	1.00	0.50	0.25
L103C	1.00	1.00	1.00	1.00	1.00	2.00	1.00	1.00	0.50	0.03	1.00	0.50	2.00	1.00	1.00	0.50	0.50
S105C	1.00	1.00	1.00	1.00	0.50	4.00	1.00	0.50	0.50	0.03	1.00	0.50	1.00	1.00	1.00	0.50	0.25

Abbreviations: acriflavin (ACR), proflavin (PRO), crystal violet (CV), rhodamine 6G (R6G), pyronin Y (PY), ethidium bromide (ET), tetraphenylarsonium chloride (TPA), methyltriphenyl phosphonium bromide (MTP), tetraphenylphosphonium chloride (TPP), dequalinium chloride (DQ), methyl viologen dichloride (MV), benzalkonium chloride (BZ), myristalkonium chloride (MAC), cetylpyridinium chloride (CPC), cetylpyridinium bromide (CPB), cetalkonium chloride (CAC), and cetrimide (CET).

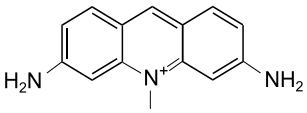
*EmrE variant fold change MIC values for STAC are omitted since there were no differences between background (empty vector) and pEmrE transformed MIC data (discussed in the Methods section).



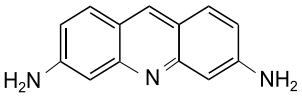
Rhodamine 6G (R6G)



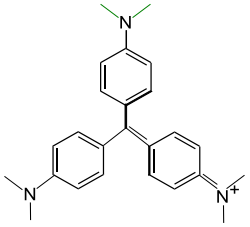
Pyronine Y (PY)



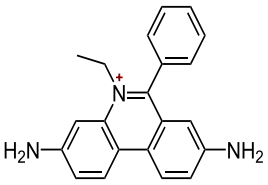
Acriflavin (ACR)



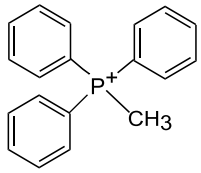
Proflavin (PRO)



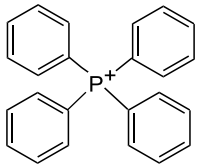
Crystal violet (CV)



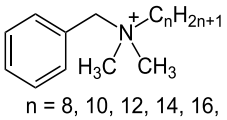
Ethidium bromide (ET)



Methyltriphenyl-
phosphonium (MTP)

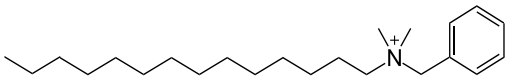


Tetraphenyl-
phosphonium (TPP)

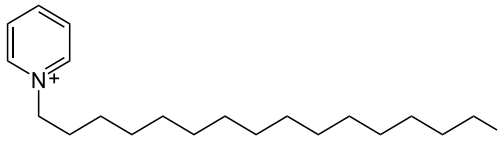


n = 8, 10, 12, 14, 16, 18

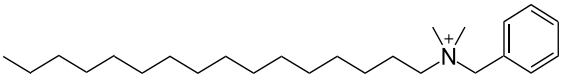
Benzalkonium (BZ)



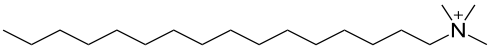
Myristalkonium (C14) chloride (MAC)



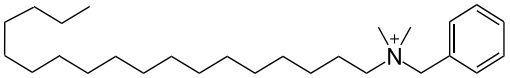
Cetylpyridium chloride (CPC)/
Cetylpyridium bromide (CPB)



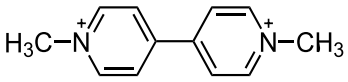
Cetalkonium (C16) chloride (MAC)



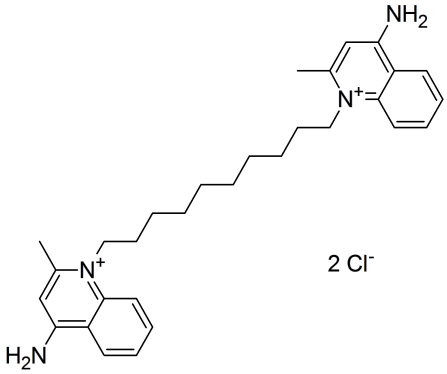
Cetrimide C8-C18 (CET)



Stearylalkonium (C18) chloride (STA)



Methylviologen (MV)

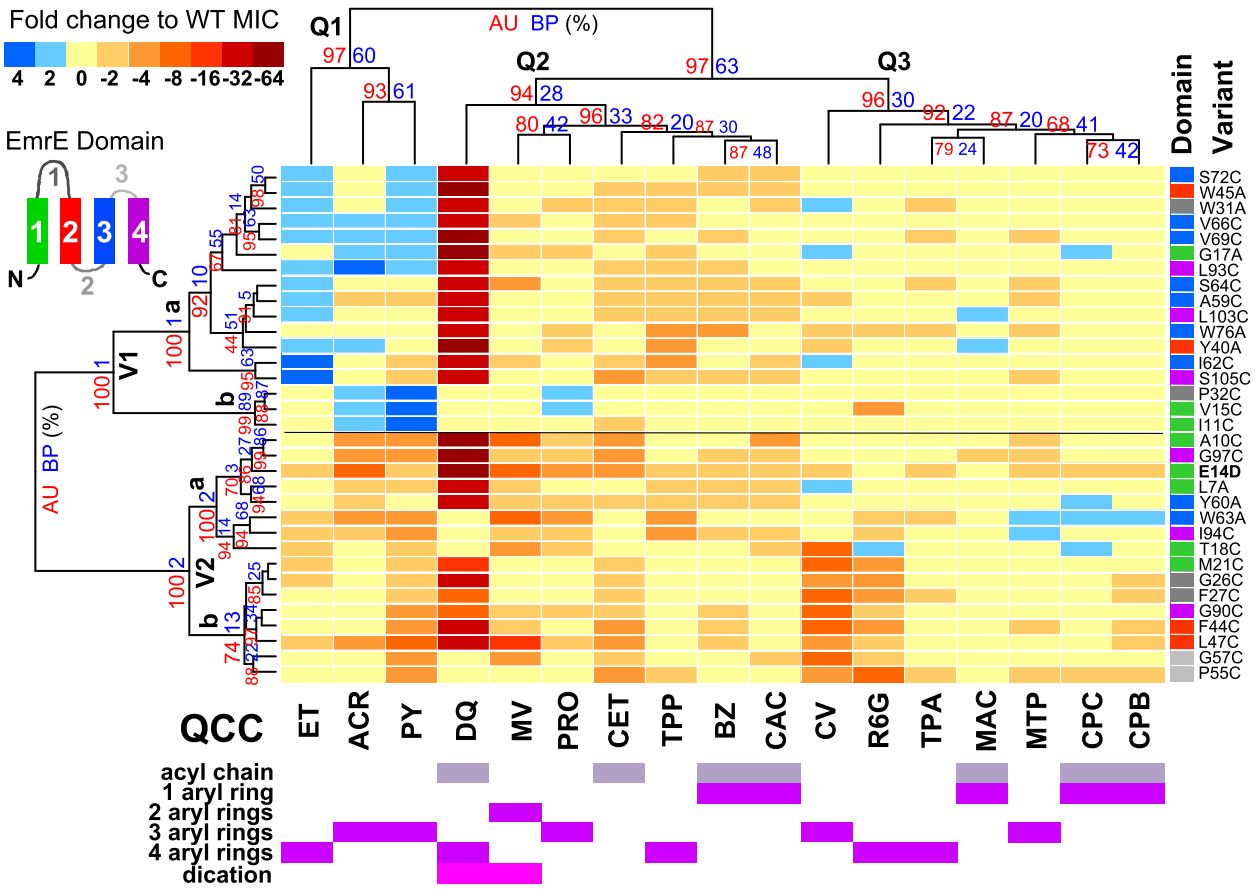


2 Cl-

Dequalinium (DQ)

Figure S1. Chemical structures of all 18 QCCs examined in this study.

Figure S1.



A hierarchical agglomerative cluster heatmap of EmrE variant MIC values determined for 17 different QCCs. The fold change in MIC for values obtained for EmrE variants (y-axis) exposed to each QCC tested (x-axis) is shown on the heatmap (refer to panel legend). The black dividing line on the heatmap indicates the division between EmrE variant clusters V1 and V2 and lettering indicates nodes discussed in text. Each EmrE variant is listed on the right hand y-axis and coloured according to its TM position. All 17 QCCs are listed on the bottom x-axis along with corresponding heatmap (shades of purple) that indicates specific chemical structural features for each drug. Dendrograms on the left y-axis and top x-axis show cluster associations between EmrE variant MICs and QCCs and clusters discussed in text are labelled according to cluster: EmrE variant clusters (V1-V2) and QCC clusters (Q1-Q3).

Figure S2

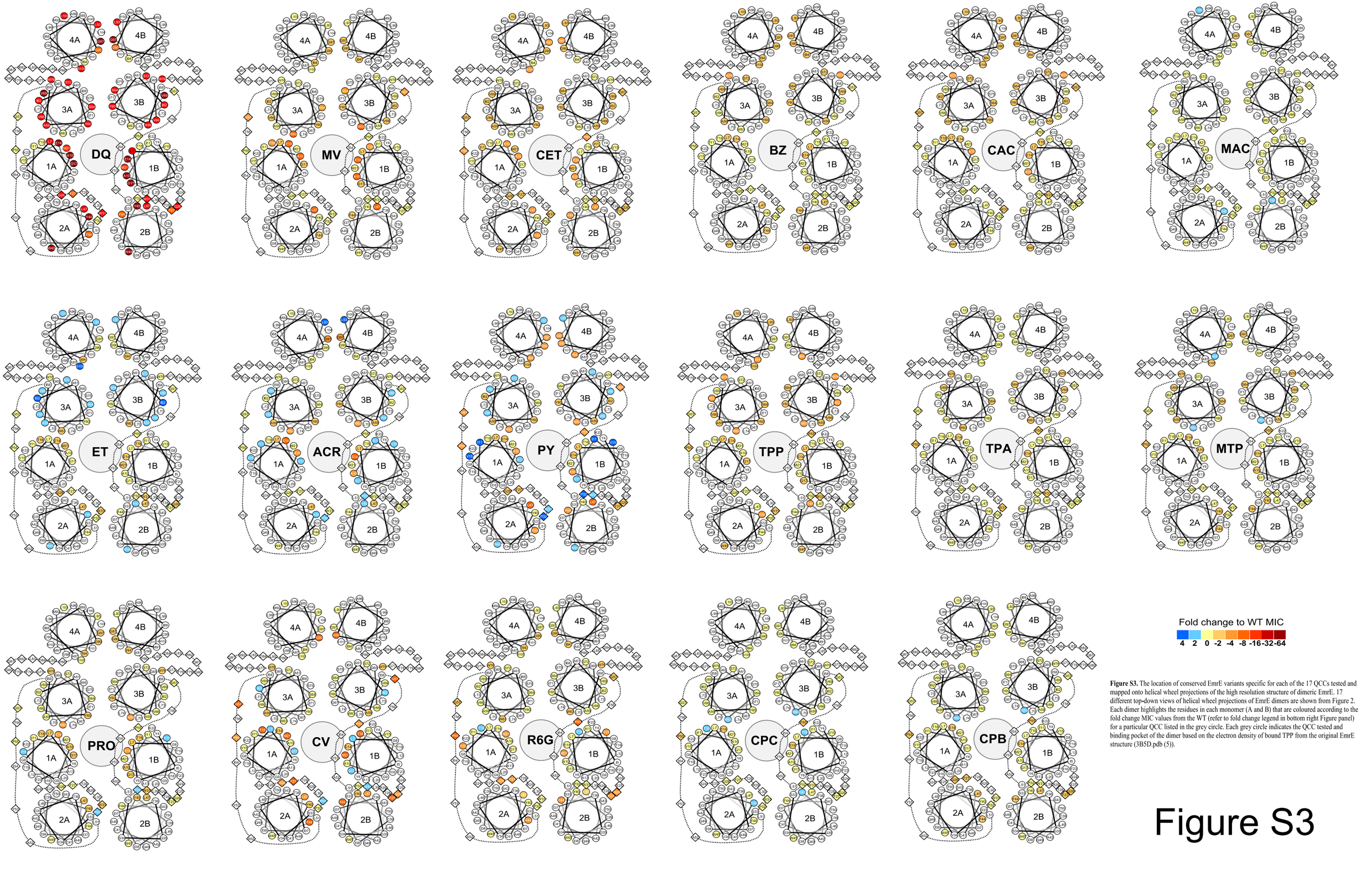


Figure S3. The location of conserved EmrE variants specific for each of the 17 QCCs tested and mapped onto helical wheel projections of the high resolution structure of dimeric EmrE. 17 different top-down views of helical wheel projections of EmrE dimers are shown from Figure 2. Each dimer highlights the residues in each monomer (A and B) that are coloured according to the fold change MIC values from the WT (refer to fold change legend in bottom right Figure panel) for a particular QCC listed in the grey circle. Each grey circle indicates the QCC tested and binding pocket of the dimer based on the electron density of bound TPP from the original EmrE structure (3B5D.pdb (5)).

Figure S3

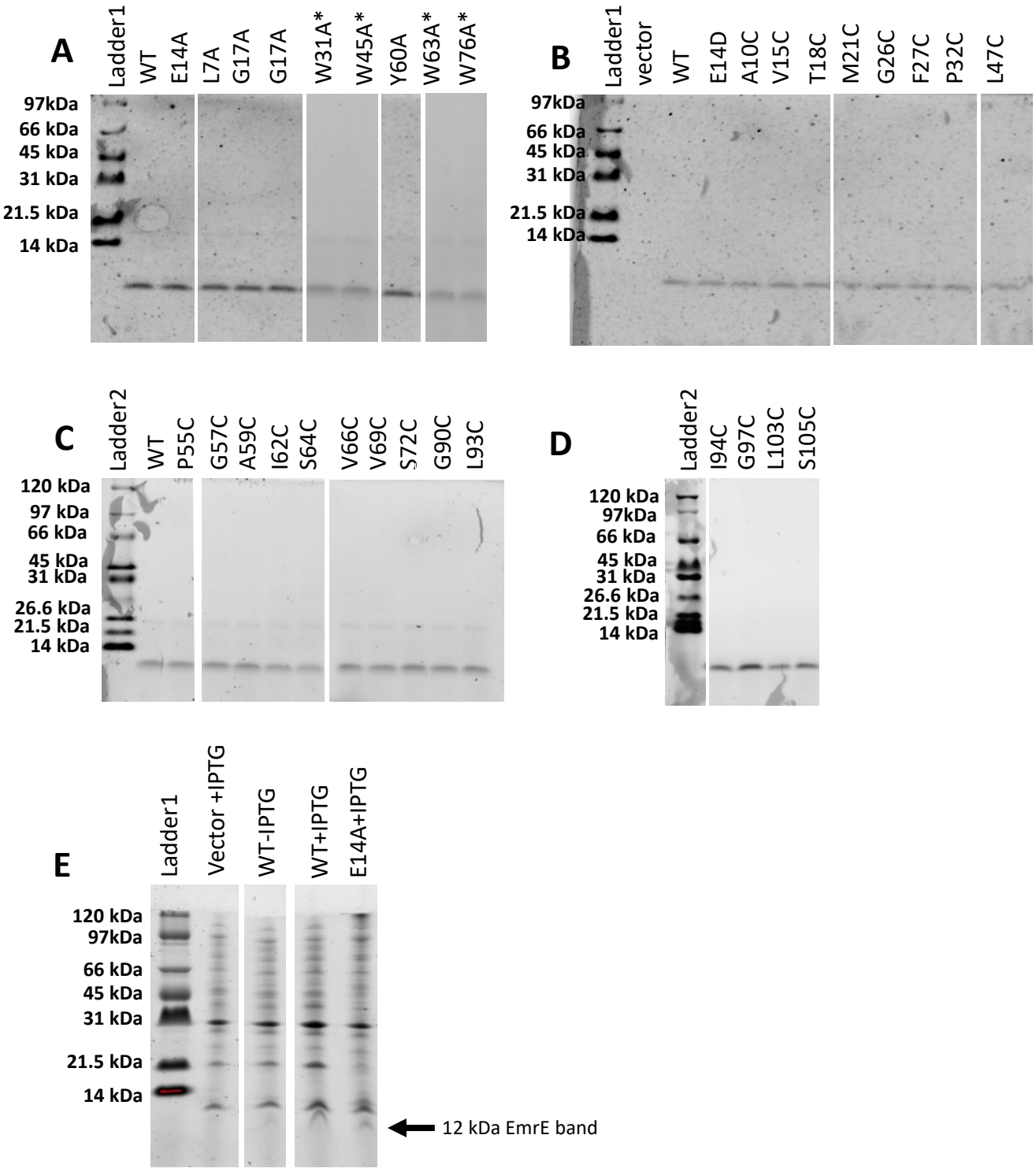

A
Ladder1
WT
E14A
L7A
G17A
G17A
W31A*
W45A*
Y60A
W63A*
W76A*
97kDa
66 kDa
45 kDa
31 kDa
21.5 kDa
14 kDa
B
Ladder1
vector
WT
E14D
A10C
V15C
T18C
M21C
G26C
F27C
P32C
L47C
97kDa
66 kDa
45 kDa
31 kDa
21.5 kDa
14 kDa
C
Ladder2
WT
P55C
G57C
A59C
I62C
S64C
V66C
V69C
S72C
G90C
L93C
120 kDa
97 kDa
66 kDa
45 kDa
31 kDa
26.6 kDa
21.5 kDa
14 kDa
D
Ladder2
I94C
G97C
L103C
S105C
120 kDa
97kDa
66 kDa
45 kDa
31 kDa
26.6 kDa
21.5 kDa
14 kDa
E
Ladder1
Vector +IPTG
WT-IPTG
WT+IPTG
E14A+IPTG
120 kDa
97kDa
66 kDa
45 kDa
31 kDa
21.5 kDa
14 kDa
12 kDa EmrE band

Figure S5.